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# Impaired slow wave sleep downscaling in patients with infantile spasms

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ABSTRACT

*Background:* West syndrome is a severe epileptic encephalopathy of infancy, characterized by infantile spasms, global retardation, and a severely abnormal electroencephalogram (EEG) pattern known as hypsarrhythmia, which is most prominent during slow waves sleep. The restorative function of slow wave sleep has been linked to downscaling, a neuronal process ensuring a balance of global synaptic strength, which is important for normal cortical functioning and development. A key electrophysiological marker for this downscaling is the reduction of the slope of slow waves across the night.

*Methods:* We retrospectively compared the slope of slow waves between 14 untreated patients with infantile spasms and healthy age and gender matched controls. Patients were examined in one all-night sleep EEG before treatment, and in two follow-up nap recordings, under and after treatment with corticosteroids.

*Results:* In patients with infantile spasms the overnight reduction in the slope of slow waves was significantly diminished compared to controls ($p = 0.009$). Moreover, untreated patients revealed overall steeper slopes. During corticosteroid treatment the slope was reduced compared to controls ($p = 0.001$). After successful treatment the slope was similar between patients and controls.

*Conclusion:* Our results provide evidence for reduced downscaling in patients with infantile spasms. Moreover, the marked reduction of the slope during corticosteroid treatment may reflect a loss of synaptic connections due to the effect of glucocorticoids. This altered sleep dependent regulation of synaptic strength in infantile spasms may contribute the underlying pathomechanism of the developmental regression. Furthermore the normalization of synaptic strength due to corticosteroids might provide a potential mechanistic explanation for this treatment strategy.



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## 1. Introduction

West syndrome is one of the most malignant epilepsies of infancy, characterized by infantile spasms, hypsarrhythmia and frequent mental retardation.[1,2] The long-term prognosis is often poor, although hormone therapy (i.e., adrenocorticotropic hormone (ACTH) or oral corticosteroids) or vigabatrin (VGB) might effectively treat the spasms and often also improve or normalize the electroencephalogram (EEG). The onset of spasms is frequently associated with developmental regression and long-term cognitive impairments. West syndrome is classified as an epileptic encephalopathy[3] indicating that the epilepsy and the abnormal EEG (hypsarrhythmia) contributes to deterioration of cerebral functioning. Although developmental outcome in infantile spasms is substantially determined by the underlying disease, evidence is accumulating that the severity of the hypsarrhythmia[4] and the time to treatment[5] is related to the developmental outcome. However, so far no mechanistic explanation of such a link exists.

Under physiological conditions deep NREM sleep is characterized in the EEG by the predominant occurrence of high amplitude slow wave. During the last decades a growing body of research has demonstrated, that these slow waves can be used as an indirect but reliable marker of synaptic strength.[6–8] Moreover, within the framework of the synaptic homeostasis hypothesis the restorative function of slow wave sleep has been directly linked to neuronal recovery and cortical maturation.[9] The hypothesis claims that the learning related rise in synaptic strength needs energy and space, eventually leading to a saturation of learning capacity. Hence, for a normal physiological development synaptic strength needs to be reduced (synaptic downscaling), which, according to the hypothesis, takes place during slow wave sleep. This global synaptic downscaling enables new cortical plasticity the next day and thereby increases learning capacity.[9] Synaptic downscaling, the renormalization of synaptic strength across the night, is reflected in the physiological decrease of the slope of slow waves across the night. Consequently, the slopes of slow waves at the beginning of the night are much steeper compared to the slopes of slow waves with the same amplitude towards the end of the night.[8]

Considering the severely disturbed slow wave sleep in patients with infantile spasms due to hypsarrhythmic pattern and the fact that the onset of spasms is often associated with mental retardation we were asking the question whether synaptic downscaling might be impaired in patients with infantile spasms. To test these hypotheses, we performed a retrospective case-controlled study in 16 infants diagnosed with West syndrome. We analyzed the characteristics of sleep slow waves (in particular the slope) in one all-night sleep EEG before treatment, and in two follow-up nap recordings (under and after treatment). Data were compared to healthy age and gender matched controls. We hypothesize that hypsarrhythmia during NREM sleep might impair the overnight reduction of the slope of slow waves in infantile spasms. Moreover, when slow wave sleep is normalized, after successful treatment with corticosteroids, we expect normalization in the slope of slow waves.

## 2. Methods

### 2.1. Participants

In a retrospective analysis untreated patients diagnosed with infantile spasms were selected from the data base of the Children's Hospital Zurich (examined between 2008 and 2012) according to the following clinical and electrophysiological criteria:

1. Onset of infantile spasms between 1 and 24 months of age
2. Infantile spasms recorded by video-EEG
3. Hypsarrhythmic pattern as the prominent EEG feature during NREM sleep and ictal EEG registered during infantile spasms
4. One complete all-night EEG before treatment, a first follow-up nap under treatment and a second follow-up nap after treatment with corticosteroids

Since 2008 an all-night video-EEG is part of our regular diagnostic work up in newly diagnosed infantile spasms to register the serial spasms and possible additional convulsive and non-convulsive seizures. In total, 16 infants met the above criteria. Age ranged from 2.5 to 11.2 months (mean age 6.2 ± 0.6 months, 11 boys). One boy was excluded from the analysis because of poor quality of the overnight EEG and another one because the EEG never showed hypsarrhythmia although serial infantile spasms were regularly registered in the EEG at awakening (for an overview of the included patients see Table 1). After the initial overnight sleep EEG, patients were treated with prednisolone (PRD) alone ($n = 3$), or ACTH followed by PRD ($n = 4$) or each combined with VGB (PRD&VGB, $n = 6$; ACTH/PRD&VGB, $n = 1$, see Fig. 1 and Table 1). Eight patients (six boys, two girls) also participated in the on-going International Collaborative Infantile Spasms Study (ICISS www.bath.ac.uk/health/research/iciss). Dosage and duration of treatment corresponded to the treatment protocol of Lux et al., 2004.[10] After ~2 weeks of treatment patients received the first follow-up nap sleep EEG (Nap 1, recording time 12:50 ± 00:36). The nap EEG of all responders (no infantile spasms, disappearance of hypsarrhythmia during wake and sleep) were further analyzed (seven boys, two girls, mean age 6.4 ± 0.5 months). Non-Responders were excluded because the short sleep duration of the nap EEG did not provide enough slow waves which were not compromised by hypsarrhythmia. After completion of treatment (3.9 ± 0.5 months after the all night EEG), patients underwent a second follow-up nap sleep EEG (Nap 2, recording time 13:27 ± 00:12). One boy relapsed and one girl did not fall asleep during the nap recording and were excluded. Thus, the naps of six boys and one girl (9.6 ± 0.9 months) were analyzed (for a detailed overview see Tables 2 and 3).

Data of the patients were compared to healthy age and gender matched controls. All control infants were free of neurological or developmental disorders. EEG recordings of the control group were obtained from different sources:

Control group 1: For the overnight comparison previously presented data from longitudinal PSGs of 11 full term infants (six girls and five boys) were analyzed (for details see Jenni

**Table 1 – Overview of the patients:**

| # | Age [Mo] | Clinical findings at presentation | Magnetic resonance imaging | Hypsarrhythmia-free interval [s] | Treatment | Response to medication | EEG Nap 1 under treatment | EEG Nap 2 after treatment | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.2 | IS only | Normal | 10.4±0.6 | PRD & VGB | Yes | No Hyps<br>No SW | No Hyps<br>No SW | Normal |
| 2 | 8.2 | IS and focal seizures, minor hemiparesis right | Pontomesencephale dysplasia bilateral insular pachygyria | 4.3±0.3 | ACTH/PRD & VGB | Yes | No Hyps<br>No SW | No Hyps<br>Few SW F3 | DR, hyperactivity |
| 3 | 7.2 | IS, DR, disturbed sleep-wake rhythm | normal | 5.6±0.2 | PRD | Yes | No Hyps<br>No SW | No Hyps<br>No SW | DR |
| 4 | 9.8 | IS only | Dysplasia (right frontal) | 5.5±0.6 | PRD & VGB/others | No | | | IS, multiple seizures, DR |
| 5 | 4.4 | IS, muscle hypotonia, irritable | Normal | 3.2±0.1 | ACTH/PRD | Yes | No Hyps<br>No SW | No Hyps<br>No SW | Normal |
| 6 | 5.0 | IS, snivelling, occasionally "mentally absent" | Normal | 2.8±0.1 | PRD & VGB | Yes | No Hyps<br>No SW | No Hyps<br>No SW | Normal |
| 7 | 3.2 | IS and focal seizures | Normal | 2.6±0.1 | PRD & VGB | No | | | DR, pharmacoresistant epilepsy |
| 8 | 4.0 | IS only | Normal | 3.8±0.1 | PRD | Yes | No Hyps<br>No SW | No Hyps<br>SW P3 | Normal |
| 9 | 2.5 | IS and focal seizures | Dysplasia (left temporal and occipital) | 3.1±0.0 | PRD & VGB | No | | | DR, focal seizures |
| 10 | 5.3 | IS, occasionally "mentally absent" | Normal | 3.4±0.1 | ACTH & PRD | Yes | No Hyps<br>No SW | No Hyps<br>No SW | DR |
| 11 | 5.3 | IS, only | Normal | 8.8±2.6 | PRD | Yes | No Hyps<br>No SW | no Hyps<br>no SW | Normal |
| 12 | 6.4 | IS, DR, muscle hypotonia, apathetic | Frontal atrophy | 5.0±0.2 | PRD | No | | | DR, muscle hypotonia |
| 13 | 7.5 | IS, only | Normal | 3.4±0.1 | PRD & VGB | Yes/relapse | No Hyps<br>Few SW T3 | | Relapse IS, DR |
| 14 | 8.6 | IS, only | Altered ventricle configuration | 5.6±0.3 | ACTH & PRD | No | | | DR, focal seizures |

Clinical findings at presentation, MRI findings, mean duration of the hypsarrhythmia-free interval, treatment, response to medication, EEG at Nap 1 (under treatment), EEG at Nap 2 (after treatment), and outcome of all patients (IS = infantile spasms, DR = developmental regression, Hyps = hypsarrhythmia, SW = spike/spike waves, VGB = vigabatrin, PRD = prednisolone, ACTH = tetracosactide depot).

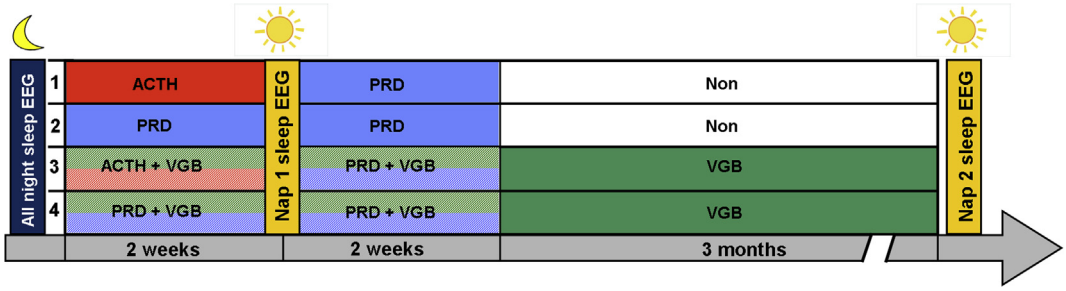


**Fig. 1 – Overview of the EEG registrations with respect to the treatment plan. After the diagnosis of infantile spasms (according to the overnight sleep EEG) patient were treated with one of four different therapy approaches. All patients received a first follow-up nap (Nap 1) after ~2 weeks of treatment. After ~4 months (discontinuation of all medications) the second follow-up nap (Nap 2) took place (ACTH = tetracosactide depot, PRD = prednisolon, VGB = vigabatrin).**

et al., 2004)[11]. To achieve good matching with the patient group we selected the overnight recordings from four boys and six girls. From two boys we used three subsequent nights. These lead to a total of 14 overnight recordings for the comparison.

Control group 2 and 3: To match the nap EEG of the patients with infantile spasms (Table 3) we selected nap EEG recordings from 15 infants (two girls and 13 boys) performed at the University Children's Hospital Zurich (1995–2012) for diagnostic routine procedures. These infants were referred to our epilepsy centre because of paroxysmal events of unknown causes. None of the infants suffered from epilepsy or seizures and none was treated with anticonvulsants or any other drug. These controls were divided into two groups (control group 2 and control group 3) corresponding to the patients age at the time of the nap recordings. Control group 2 included nine infants (seven boys and two girls, mean age 6.6 ± 0.4 months) recorded on average at 12:27 ± 00:43. Control group 3 consisted of seven infants (six boys and one girl, mean age 9.5 ± 1.0 months) recorded on average at 13:22 ± 00:33. The nap recording of one girl was used twice i.e. in the control group 2 and 3.

The study was conducted at the University Children's Hospital Zurich (Switzerland) and approved by the local ethics committee. The study was performed according to the Declaration of Helsinki.

### 2.2. *EEG recordings*

All PSG of the patients and the control groups 2 and 3 were obtained by Neurofile/Coherence (Natus Europe GmbH, Munich, Germany). The EEG recordings included at least 21

**Table 2 – All-night sleep.**

| | Age [d] | Sex | TST [min] | NREMS [min] | REMS [min] | Wake [%] | WASO [min] |
|---|---|---|---|---|---|---|---|
| Control group 1 | 177.9 ± 17.7 | 8m*/6f | 518.4 ± 25.1 | 300.8 ± 19.3 | 187.4 ± 11.1 | 9.5 ± 1.3 | 37.5 ± 7.2 |
| Patients overnight | 181.5 ± 17.4 | 9m/5f | 486.8 ± 15.3 | 317.2 ± 12.6 | 131.5 ± 3.7 | 10.6 ± 1.2 | 52.2 ± 7.1 |
| *p*-Value | 0.44 | | 0.15 | 0.24 | <0.001 | 0.29 | 0.08 |

Age, gender and sleep architecture of the overnight recordings for the control group 1 and the patients. Mean values ± SE are shown (TST = total sleep time (between the first and the last hour of NREM sleep); NREMS = NREM sleep; REMS = REM sleep; Wake = percentage of wakefulness for the defined night sleep period; WASO = wake after sleep onset, * from 2 boys 3 different night recordings are used).

**Table 3 – Nap 1 & 2.**

| | Age [d] | Sex | RT [min] | TST [min] | NREMS [min] | REMS [min] |
|---|---|---|---|---|---|---|
| Control group 2 | 200.8 ± 12.4 | 7m/2f* | 11.4 ± 42.7 | 25.5 ± 2.3 | 18.3 ± 1.9 | 0 ± 0 |
| Patients Nap 1 | 195.6 ± 14.9 | 7m/2f | −11.4 ± 36.5 | 26.5 ± 2.3 | 23.2 ± 2.2 | 0.7 ± 0.7 |
| *p*-Value | 0.4 | | 0.28 | 0.38 | 0.06 | 0.17 |
| Control group 3 | 390.0 ± 30.8 | 6m/1f* | 2.3 ± 33.5 | 22.1 ± 1.8 | 18.5 ± 1.8 | 0 ± 0 |
| Patients Nap 2 | 291.4 ± 28.4 | 6m/1f | −2.3 ± 11.5 | 23.8 ± 1.8 | 20.7 ± 2.4 | 0 ± 0 |
| *p*-Value | 0.49 | | 0.4 | 0.26 | 0.23 | 1 |

Age, gender and sleep architecture of the nap recordings for the control group 2 & 3 and the patients (Nap 1 & 2). Mean values ± SE are presented (RT = recording time (start time of the recording expressed as difference to the mean start time across the control group 2 and Nap 1 (12:38) and across the control group 3 and Nap 2 (13:24); TST = total sleep time, NREMS = NREM sleep, REMS = REM sleep; *the nap recording of one girl was used for control group 2 & 3).

channels according to the 10–20 system (FP1, FP2, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, T3, T4, T5, T6, FZ, CZ, PZ, A1, and A2, referenced to F3 or F4 or to an additional electrode between FZ and CZ). In addition, EMG and EOG were used to score sleep stages. If EMG or EOG were not included in the montage, EMG from the neck muscles (O1–O2) and EOG from the prefrontal electrodes (FP1–FP2) were used. The original EEG signal was sampled at 256 Hz. After band-pass filtering (0.16–40 Hz) the EEG was downsampled to 128 Hz. Overnight EEGs of the control group 1 were recorded with a portable polygraphic amplifier system (PS1; Institute of Pharmacology and Toxicology, University of Zurich, Switzerland). The electrodes were placed along the antero–posterior axis over both hemispheres according to the 10–20 system (bipolar derivations: F3C3, F4C4, C3P3, C4P4, P3O1, P4O2; the signal was recorded to the reference derivation C3A2). The signals were sampled at 512 Hz (0.16–70 Hz), low-pass filtered (below 30 Hz) and downsampled to 128 Hz (for details see Jenni et al., 2004)[11].

Sleep stages were visually scored for consecutive 20-s epochs. For the healthy control groups and the nap EEGs of the patients (Nap 1 & 2) sleep stages were determined according to standard criteria (NREM sleep 1–3, REM). Overnight recordings of the patients were difficult to score according to standard criteria because of the pathological electrophysiological changes. Therefore criteria were adapted (based on suggestions of Landolt et al., 2006)[12] similar to the ones described by Bölsterli et al., 2011.[13] Only three different sleep stages were scored: wakefulness, NREM sleep, and REM-like sleep. The analysis of sleep slow waves of the current work was based on NREM sleep (healthy controls N2 & N3 combined, patients NREM sleep). Therefore, the missing subdivision into N2 and N3 in the patients did not influence the results.

### 2.3. *Data analysis*

The analysis was based on the EEG derivation P3A2. However, data from the other derivations revealed similar results (data not shown); therefore only data from P3A2 are presented. Thus, the EEG signal over the left parietal cortex was re-referenced to the right mastoid (P3A2). Visual and semi-automatic artefact correction was performed based on two frequency bands (0.75–4.5 Hz and 20–30 Hz)[14] for the removal of epochs containing poor data quality.

The physiological decrease of the slope of slow waves across the night is already present at the age of 2 months.[15] Therefore, single sleep slow waves were detected, to analyze the overnight decrease of the slope of slow waves as a marker of synaptic downscaling. Spike waves and slow frequency activity in infantile spasms patients are likely to reflect altered neuronal activity of cortical neurons. Therefore, only normally shaped sleep slow waves should be analyzed to achieve a reliable comparison between infantile spasms patients and healthy controls.

Since we were interested in overnight changes of synaptic strength, the beginning and the end of the all-night sleep EEG of all patients were carefully inspected by a certified EEG specialist (BS). The night sleep period was defined from the first to the last scored NREM sleep epoch. The night sleep period was considered terminated when the last NREM sleep cycle was followed by more than one consecutive hour of wakefulness (minimal sleep period in the patient group 407.7 min, in the control group 391.3 min). Only artefact free NREM epochs consisting of EEG activity without spike waves or hypsarrhythmia and lasting for at least 2 s (for mean duration of the hypsarrhythmia-free intervals of each patient see Table 1) were marked until ~15 min of normal NREM sleep EEG activity at the beginning and the end of the overnight EEG was collected. For the further analysis of the slope of sleep slow waves only the marked epochs were considered. Inspection of the raw EEG revealed that 15 min of normal sleep EEG were sufficient to obtain enough slow waves to perform our analysis. On average 52.1 ± 3.6 min of NREM sleep needed to be inspected to obtain 15 min of normal NREM sleep EEG. Within these 15 min of normal NREM sleep EEG, 611.3 ± 51.3 sleep slow waves were detected (for detection algorithm see below). To assess overnight changes in the slope of sleep slow waves of healthy controls single slow waves of the first and last hour of NREM sleep were detected (on average 2280.8 ± 67.6 sleep slow waves were detected). For reasons of simplification, the term first and last hour of NREM sleep is used for both groups to describe the beginning and the end of sleep. The nap EEGs of all patients were also inspected, spike wave activity was marked and excluded from the analysis.

Next, single slow waves were automatically detected applying a similar procedure as described by Riedner et al. (2007)[8]: After band-pass filtering (Chebyshev Type two Filter: pass-band 0.5 and 4.0 Hz, stop-band: below 0.16 and above 10 Hz) sleep slow waves during artifact free NREM sleep epochs (i.e. stage ≥NREM2) were detected as negative deflections of the EEG signal between two consecutive zero-crossings. For all negative half-waves of any amplitude but within a frequency of 0.5 and 2 Hz the samples of all zero-crossings and local minima of the signal (amplitude) were identified. The ascending slope of slow waves was then defined as the amplitude divided by the time difference between the sample of the local minimum and the subsequent zero-crossing (for a schematic illustration see Fig 1 in[16]).

The slope and the amplitude of sleep slow waves are closely related.[8] The larger the amplitude of slow waves the steeper the slope. Therefore, for an unbiased assessment of synaptic strength, general amplitude differences need to be controlled. To take this into account the slope of sleep slow waves with different amplitudes were analyzed separately. The mean slope of slow wave of all detected waves within 10 μV amplitude bins was calculated for each subject. Only amplitude bins containing at least 20 waves were considered.

### 2.4. *Statistics*

For the comparison of the slope of slow waves across different amplitude bins between patients and healthy controls a linear mixed model ANOVA was calculated with subjects as random intercept (to account for repeated measurements of the same person), group/time and amplitude bins as fixed effects. Duration (to control for different time intervals between the first and last detected wave) and any sleep variable revealing group differences, were included as covariance in the model. If covariates were not significant within the model, they were excluded to report main effects. Paired or unpaired student t-tests (one sided) were used to compare intra- and inter-

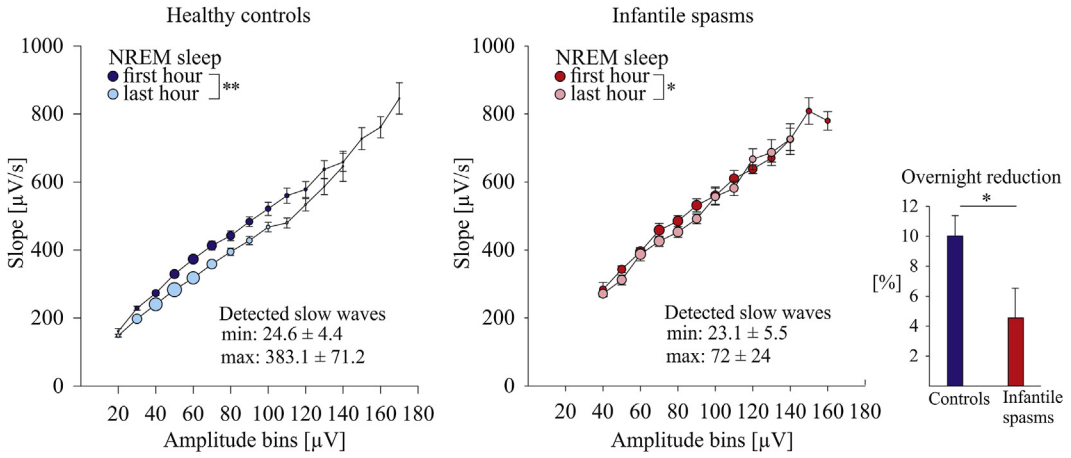


**Fig. 2 – Slope of sleep slow waves for consecutive 10 μV amplitude bins (numbers represent the upper limit) of the first and last hour of NREM sleep for healthy controls (left) and patients with infantile spasms (middle). Group mean ± SE for both time points are shown. Circle size represents the number of slow waves detected for each amplitude bin. Numbers in the lower right corner of each plot show the maximal and minimal number of slow waves detected across all subjects (mean ± SE). Note the general lower amount of detected waves in the patient group (ANOVA fixed effect "Time" (first hour vs. last hour) only between overlapping amplitude bins, $n \geq 7$, $^{**}p < 0.001$, $^{*}p = 0.009$). Right: average decrease of the slope of slow waves for healthy controls (blue) and infantile spasms patients (red; group mean ± SE, unpaired student *t*-test, $^{*}p = 0.037$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)**

individual differences. The significance level was set at 5%. All data are presented as mean ± standard errors [SE]. All analyses were performed using the software package MATLAB (MathWorks) or SPSS 16.0 (IBM).

## 3. Results

### 3.1. Sleep architecture

Sleep architecture of the overnight recordings was comparable between the healthy controls (control group 1) and patients with infantile spasms (Table 2). Both groups showed adequate sleep quality, with only ~10% wakefulness for the considered night sleep period (for details see methods). The only difference in sleep parameters was found in the amount of REM sleep: healthy controls spent more time in REM sleep compared to patients. During the nap recordings (Table 3), no significant differences were found between patients and controls.

### 3.2. Overnight changes of sleep slow waves

To investigate overnight changes in sleep slow waves, single slow waves were detected during the first and last hour of NREM sleep (i.e., NREM stage 2 and 3, for details see Methods). The results of the following paragraph include comparisons between the overnight recordings of the patients and control group 1.

The slope and the amplitude of slow waves are closely related, the larger the amplitude the steeper the slope.[8] Meaning, for an unbiased assessment of the slope of slow waves the amplitude needs to be controlled. Therefore the slopes of slow waves with the same amplitude were calculated.

In the healthy controls we found a consistent overnight decrease of the slope of slow waves from the first to the last hour of NREM sleep, independent of the amplitude (ANOVA $F_{Time} = 96.2$, $p < 0.001$; Fig. 2 left panel). We also found an overnight decrease in the patient group (ANOVA $F_{Time} = 6.9$, $p = 0.009$; Fig. 2 middle panel). However, this overnight decrease was significantly smaller in patients as compared to control subjects (ANOVA $F_{Group} = 7.9$, $p = 0.009$). The slope decreased by 10.0 ± 1.4% on average in controls and by 4.5 ± 2.1% in patients (Fig. 2, right panel).

During the first hour of NREM sleep the slope of slow waves tended to be steeper in patients compared to controls (ANOVA $F_{Group} = 3.5$, $p = 0.07$; Fig. 3 left panel). Due to the impaired overnight decrease of the slow wave slope in patients, this difference reached significance when comparing the slope of slow waves of the last hour of NREM sleep (ANOVA $F_{Group} = 16.9$, $p < 0.001$). These findings were not affected by the different number of detected waves between the controls and the patients. When we calculated the slope of 282 (minimal number of detected waves in the patients) slow waves (randomly selected) of the first and last hour of NREM sleep, a similar result was found (first hour: ANOVA $F_{Group} = 4.3$, $p = 0.048$; last hour: ANOVA $F_{Group} = 45.4$, $p < 0.001$).

### 3.3. Effect of treatment on the slope of slow waves

After 2 weeks of treatment the first follow-up nap sleep EEG (Nap 1) was recorded. Interestingly, patients revealed less steep slopes when compared to controls for slow waves with similar amplitudes, indicating a reduction of synaptic strength under treatment (ANOVA $F_{Group} = 14.9$, $p = 0.001$,

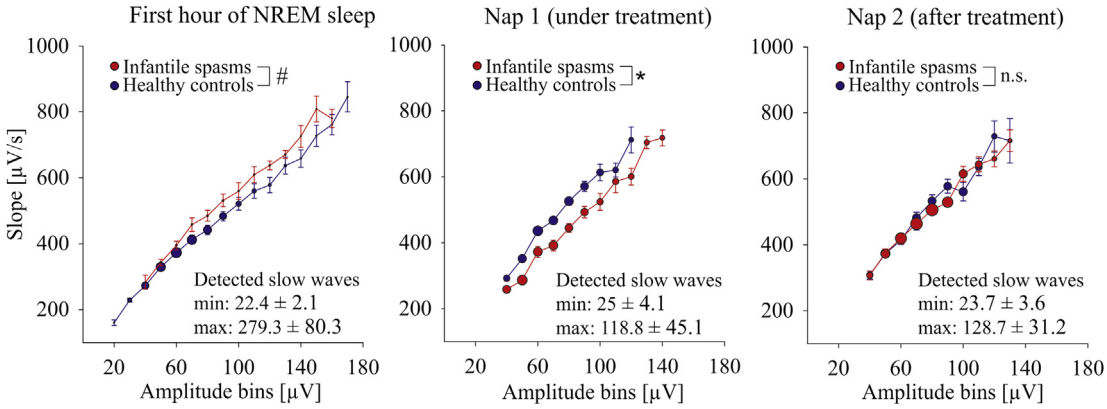


**Fig. 3 – Slope of sleep slow waves for consecutive 10 μV amplitude bins (numbers represent the upper limit) of healthy controls (blue) and patients with infantile spasms (red) for 3 different points in time (from left to right) before treatment, under treatment, after treatment. Group mean ± SE are shown. Circle size represents the number of slow waves detected for each amplitude bin. Numbers in the lower right corner of each plot show the maximal and minimal number of slow waves detected across all subjects (mean ± SE; ANOVA fixed effect Group (healthy controls vs. infantile spasms) only between overlapping amplitude bins; $n \geq 5$, $^{*}p = 0.001$, $\#p = 0.07$, n.s. = not significant). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)**

Fig. 3 middle panel). Since the duration spent in NREM sleep tended to be longer in patients compared to controls (Table 3), we also included the amount of NREM sleep as a covariate in the model. However, the difference in the slope between patients and control could not be explained by a longer duration of NREM sleep found in patients (ANOVA $F_{NREM} = 0.01$, $p = 0.98$).

A second follow-up nap EEG (Nap 2) after treatment (~4 months after the diagnosis of infantile spasms) was recorded and again compared to healthy controls (control group 3). No differences were found in the slope of sleep slow waves between controls and patients, (ANOVA $F_{Group} = 0.2$, $p = 0.7$, Fig. 3 right panel).

## 4. Discussion

The physiological decrease of the slope of slow waves across the night, reflecting the restorative function of sleep, was reduced in infants with infantile spasms compared to controls. Moreover, patients revealed steeper slopes, a sign of increased synaptic strength. However, during corticosteroid treatment the slope of slow waves was markedly reduced. After successful treatment, the slope of slow waves normalized, revealing similar values compared to controls.

### 4.1. *Selection of feasible sections of physiological slow waves during NREM sleep before treatment*

All included patients showed a typical, atypical or modified hypsarrhythmic pattern during a significant time period during NREM sleep of the all-night recordings before treatment. But, all patients also showed repeated periods of NREM sleep without overlying hypsarrhythmia or spike waves, periods in which physiological sleep rhythms became obvious. Because 12 of 14 of our patients were diagnosed within 4 weeks after initiation of spasms this "fragmented" hypsarrhythmia might be due to the short diagnostic delay. In addition, 9 of 14 of the included patients had a normal MRI and an unknown etiology. Those idiopathic or cryptogenic patients are known to show "fragmented" hypsarrhythmia, especially at the very beginning and as mentioned by Dalla Bernardina and Watanabe bilateral physiological background activity and well defined physiological rhythms during sleep are often recognizable between hypsarrhythmia.[17] Although all patients in our study have EEG registered infantile spasms and hypsarrhythmia during a significant time of NREM sleep, the density of hypsarrhythmia was different: in some patients we found NREM sleep periods without hypsarrhythmia lasting ~15 s, in other patients the hypsarrhythmia-free intervals were mostly between 2 and 3 s (for an overview of the mean duration of the hypsarrhythmia-free intervals of each subject see Table 1). Our cohort was too small to investigate the influence of hypsarrhythmia density or "spike wave index" on the overnight decrease of the slope of slow waves, as recently found in patients with CSWS.[18] One patient of our cohort was excluded, since the EEG never showed hypsarrhythmia. Interestingly, in this patient we found an overnight decrease of the slope of slow waves which was similar to the control group (9.8%).

### 4.2. *Sleep architecture*

The sleep stage variables were comparable between patients with infantile spasms and healthy controls. In the considered night sleep period of the overnight recordings both groups showed adequate sleep quality. The good sleep quality of the patients group could be explained by the relatively normal clinical presentation at the time of recording (see Table 1). Only in one patient (#3) an altered sleep-wake rhythm was

reported. However, at the recording night of this patient only 8.4% of wakefulness was scored. As reported previously, the amount of REM sleep was reduced in patients.[19] No correlations, however, were found between REM sleep and overnight slope changes, both in patients and healthy controls (data not shown).

### 4.3. *Overnight changes of sleep slow waves – before treatment*

In infantile spasms patients the decrease of the slope of slow waves across the night, a marker for the restorative function of sleep,[8] was significantly reduced, indicating impaired synaptic downscaling. So far, several links between the slope of slow waves and synaptic strength have been reported. For example, Kurth et al., 2010[16] showed that the reduction in synapse density during puberty parallels a decrease of the slope of slow waves. Moreover, the slope follows a similar trajectory as the increasing synaptogenesis during the first year of life.[15] According to the synaptic homeostasis hypothesis physiological slow wave sleep is pivotal for synaptic downscaling.[9] The underlying cortical slow oscillation between periods of neuronal activity and periods of neuronal silence[20] are functionally related to a proportional downscaling of all synapses leading to a net reduction of synaptic strength.[21,22] Thus, the chaotic pattern of hypsarrhythmia during slow wave sleep might impair synaptic downscaling in patients with infantile spasms.

### 4.4. *Effect of treatment on the slope of slow waves*

After the initial diagnosis, all patients were treated with corticosteroids (PRD or ACTH/PRD) alone or combined with VGB (PRD&VGB or ACTH/PRD&VGB) and a first follow-up nap was recorded (still under treatment). The sleep EEG of all responders was further analyzed. Interestingly, beside the elimination of hypsarrhythmia the slope of slow waves was markedly reduced. Assuming that synaptic strength is reflected in the slope of slow waves, the pronounced decrease of the slope might reflect a loss of synaptic connections due to an effect of glucocorticoids in the brain. Indeed, the potential for high dosage of glucocorticoids to induce dendritic atrophy and synapse loss in the cerebral cortex and hippocampus has been reported in animal models.[23–27] In addition, there are several case reports in the literature reporting cerebral atrophy during corticosteroid treatment. The atrophy was reversible after cessation of medication.[28–30] Since no MRIs during and after treatment are available of our patient sample, a causal relationship between cortical atrophy (as a possible consequence of synaptic loss) and reduced slope values remains speculative.

After successful treatment of hypsarrhythmia and discontinuation of all medications (after ~4 months), the slope of slow waves was similar between patients and controls indicating a normalization of synaptic strength. However, whether the normalization of the slope of slow wave is indeed related to an improved overnight synaptic downscaling in patients with infantile spasms can only be speculated, since only nap sleep EEGs were available under and after treatment. Furthermore, the different level of the homeostatically regulated sleep pressure during daytime and nighttime[31] does not allow a direct intraindividual comparison of the slope of slow waves before and during/after treatment. Moreover, it would be interesting to know, whether the regulation of the slope of slow waves was still impaired in the non-responder group. Unfortunately all non-responders had to be excluded, since the short sleep duration of the nap including hypsarrhythmia did not provide enough slow waves for the analysis.

Several neurological disorders have been associated with infantile spasms, which certainly contribute to the developmental impairments in these patients. However, besides the broad heterogeneity, all patients included in the current study, suffered from hypsarrhythmia during a significant period of NREM sleep. Moreover, the disappearance of hypsarrhythmia was associated with a normalization of the slope of sleep slow waves. The analysis of the current work was based on normal sleep slow waves (i.e., not associated with epileptiform activity). Our findings show that the slope of slow waves can be used as an electrophysiological marker to investigate signs of altered neuronal properties independent of epileptiform discharges. Thus, direct comparisons between clinical and healthy population are feasible.

### 4.5. *Limitations and outlook*

Discussing these results some additional limitations need to be considered. The low number of patients included in our study prevents any comparisons between the different treatment approaches. Thus, the influence of the treatment approaches (i.e., corticosteroids alone or combined with VGB) on synaptic homeostasis needs to be investigated in a larger sample. Furthermore, for the overnight comparison, there is an imbalance in the gender matching between control group 1 and the patient group (control group 1: 8 boys; patient group: 9 boys). Our goal was to match the patient group as close as possible regarding gender and age. Since we have shown a strong influence of age on the slope of slow waves,[15] but not gender (control group 1: unpaired Student $t$-test $p = 0.46$), our primary criterion for the matching was age and not gender. We do not believe that this imbalance has any influence on our results since no difference between boys and girls has been found.

### 4.6. *Conclusion*

In conclusion, our analysis provides evidence for impaired synaptic downscaling due to hypsarrhythmia in patients with infantile spasms. Successful treatment with corticosteroids seems to reduce synaptic strength supporting a normalized regulation in the balance of synaptic strength. Such a normalization of synaptic homeostasis seems to be essential, because it is thought to be important for brain development and cerebral functioning during the day. Proportional downscaling of all synapses may reduce energy and space demands while maintaining the relative synaptic weights and thereby increases the efficiency of neuronal functioning.[9] Hence, an impaired synaptic downscaling resulting in an altered synaptic balance might contribute to the developmental regression seen in these patients.

## Conflict of interest

None.

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